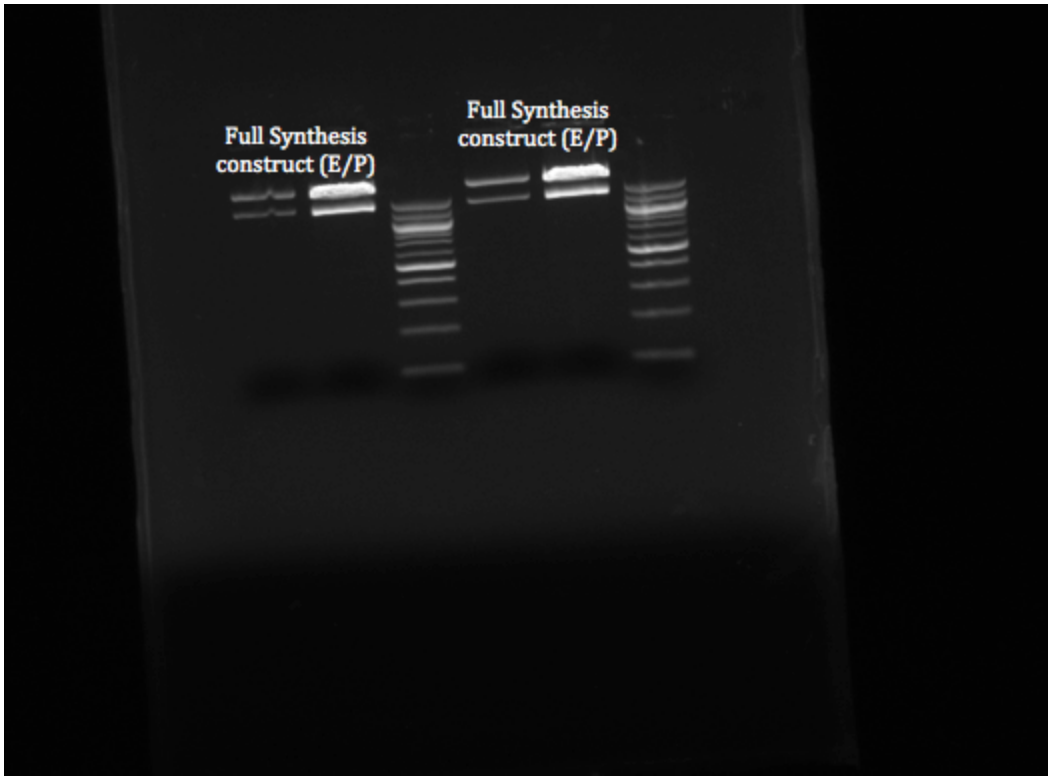
JULY

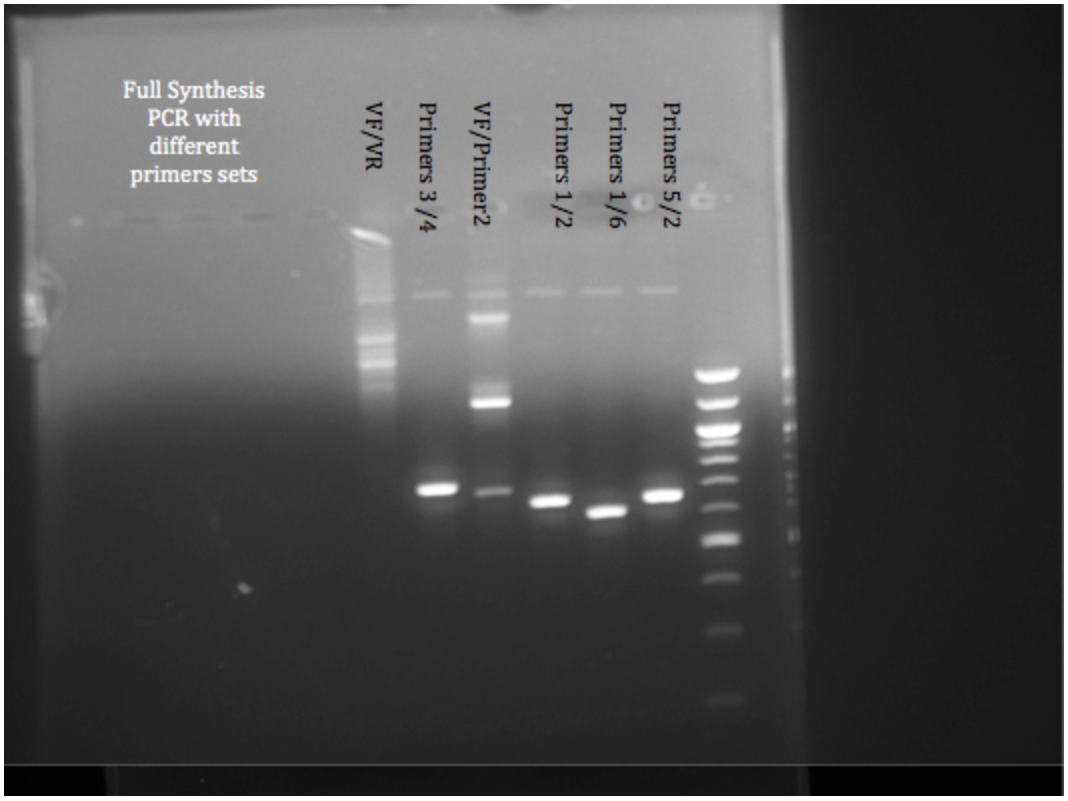
7/2/2014
- Did a colony PCR of the full construct, one with primers Vf and P2 and the other with P1 and P2
- Note that P1/P2 = 900 base pairs
- Ran a gel of the PCR product and it worked. Will do it again with DNA from the full construct miniprep.

7/3/2014
- Mini-prepped the full constructs 4.1 and 4.2
- Performed a colony PCR (using 1ul of DNA from miniprep) of 4.1 and 4.2
- Digested overnight with E/P

7/4/2014
- Ran a gel of the plasmid digest (E/P), and it turns out the plasmids are the right size



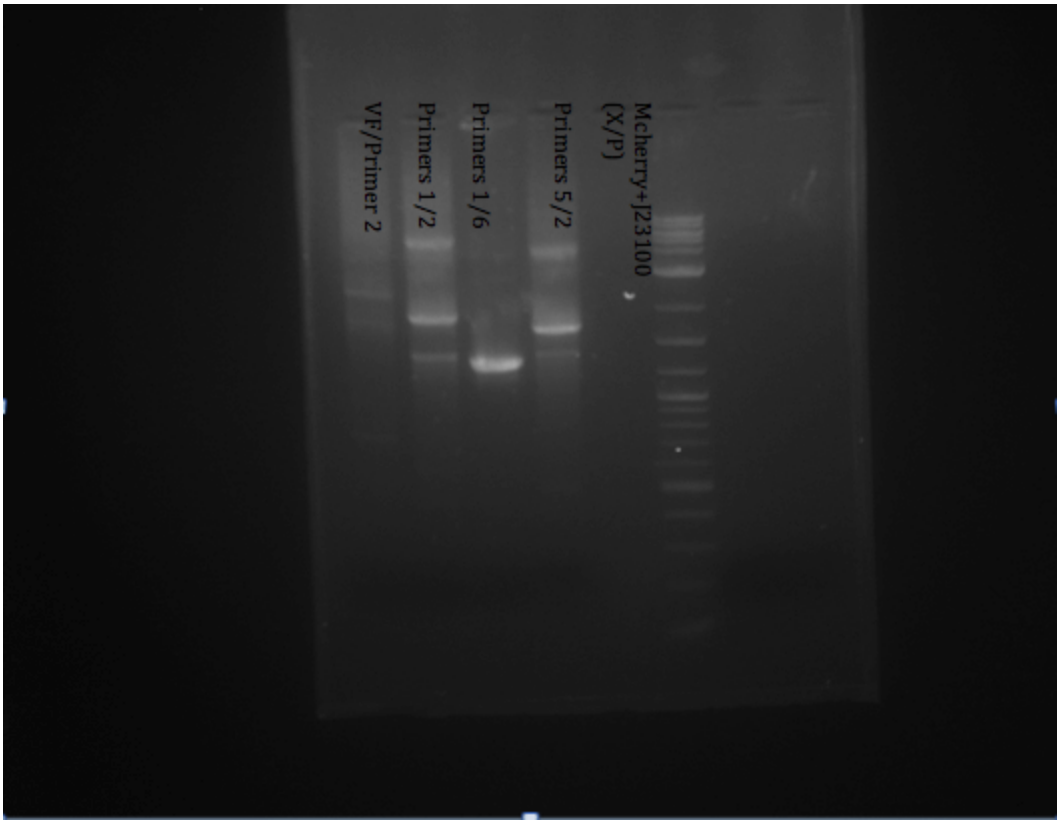
7/6/2014
- Performed a Taq. PCR for all teams and checked the annealing temperatures
- Transformed J23100+B0034 from the registry (Cm resistance)
- Ran a 1% agarose gel to confirm the sizes



- Gel extracted the 6 DNA samples
- Ligated the samples with pGEM

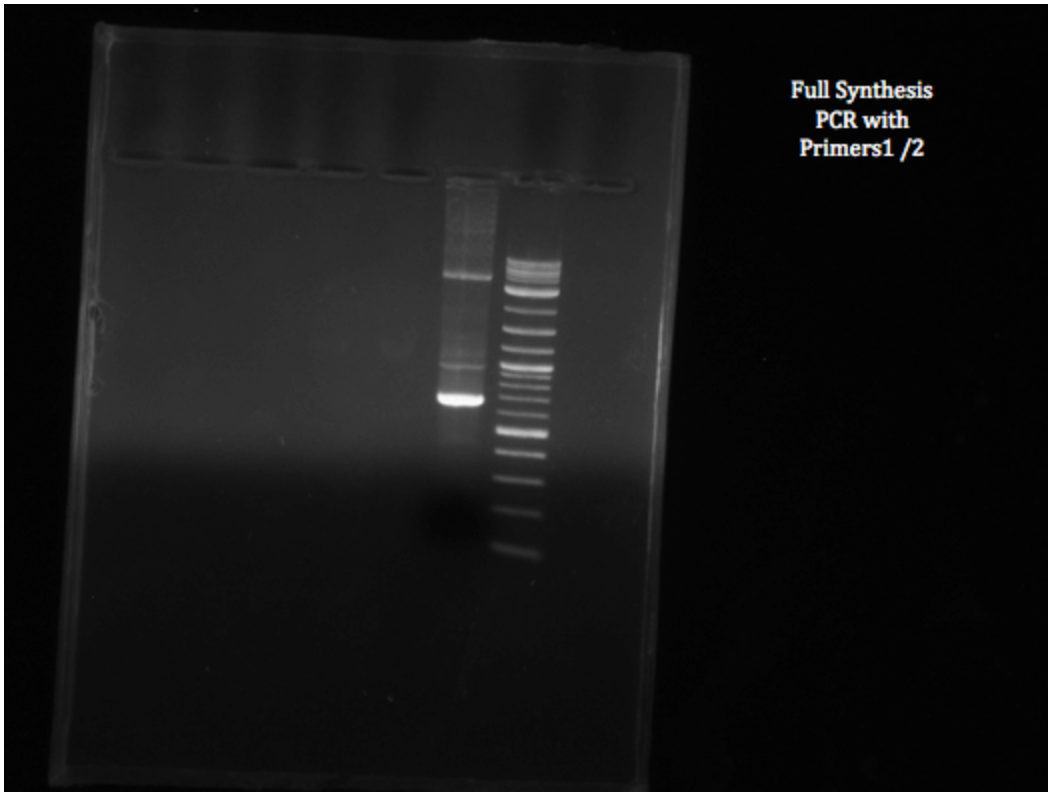
7/7/2014
- Made plates with amp resistance, IPTG and Xgal
- Made cultures of T7+RBS, MCherry
- Digested Mcherry+T23100 with X/P to see whether we got vector vector ligations or if RFP is actually in the construct

7/8/2014
-Mini-prepped T7, J23100 + B0034, & J06505 (MCherry). Placed in white freezer in Team 2's rack.
- Ran a gel of the PCR products and digest products (X/P)



- Gel extracted, PCR amplified, and ligated the DNA into pGEM (we left the ligation at room temperature for 6 hours)
- Transformed the pGEM ligations
- Mini-prepped T7, J23100 + B0034, & J06505 (MCherry)

7/10/14
-Inoculated cultures of B7.
- Mini-prepped the cultures with successful ligations for sequencing
- Our 1.1 ligation didn't grow, so we decided to do the ligation from the beginning including the PCR reaction
- Ran the PCR with Primers 1 and 2 at 50.5C
- Ran the PCR product in a 1% agarose gel, and the bands seem to be the right size



- Gel extracted and ligated the products with pGEM overnight
- Inoculated cultures of B1

7/11/14
- Mini-prepped ligation 1.1 that was grown from the replica plate
- Transformed the pGEM ligation 4.1 (P1 and P2)
- Started 2 cultures of J23100 and T7 (both with RBS) Cm resistance
- Did a PCR with P1/P2 and P1/P4. We should see a ~600 band with P1/P2 and no bands with P1/P4. The gel indicates that the ligation did not work.

7/12/14
- Mini-prepped cultures of promoters for T7 and J23100
- White colonies found on the plate of the 4.1 pGEM ligation were numbered from 1-5, grown in media, and replica plated

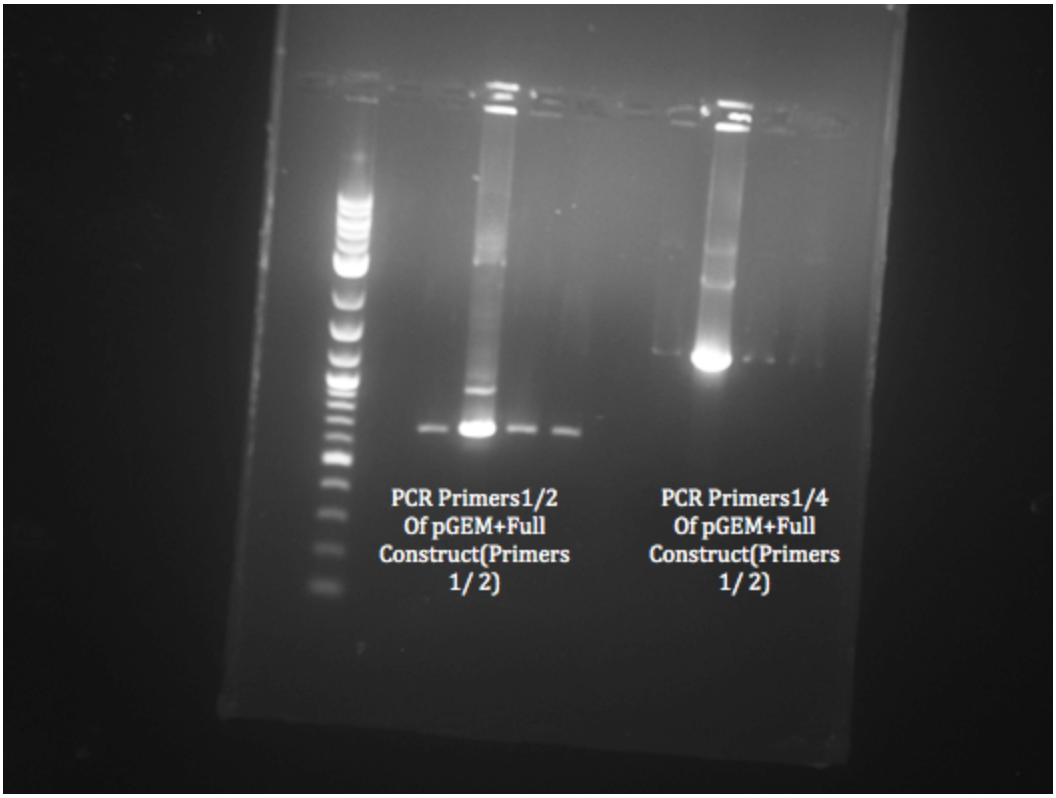
7/13/14
- Mini-prepped colonies 4.1-4.5 P1/P2 in pGEM

7/14/14
- The 4.1-4.4 minipreps were digested with X/P (we didn't use the 4.5 mini prep because a white colony grew in the replica plate)
- T7 + J23100 were digested with E/S

- Vector IK3 (kan. resistant, iGEM standard vector) was digested with E/P
- Ligated the minipreps, promoter, and IK3 overnight
- Prepared a total of twelve cultures. There were two new cultures + Ampicillin per each colony for both ligations 2 and 3. We allowed the cultures to incubate overnight.
- Labels for the cultures started: 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, and 4.2

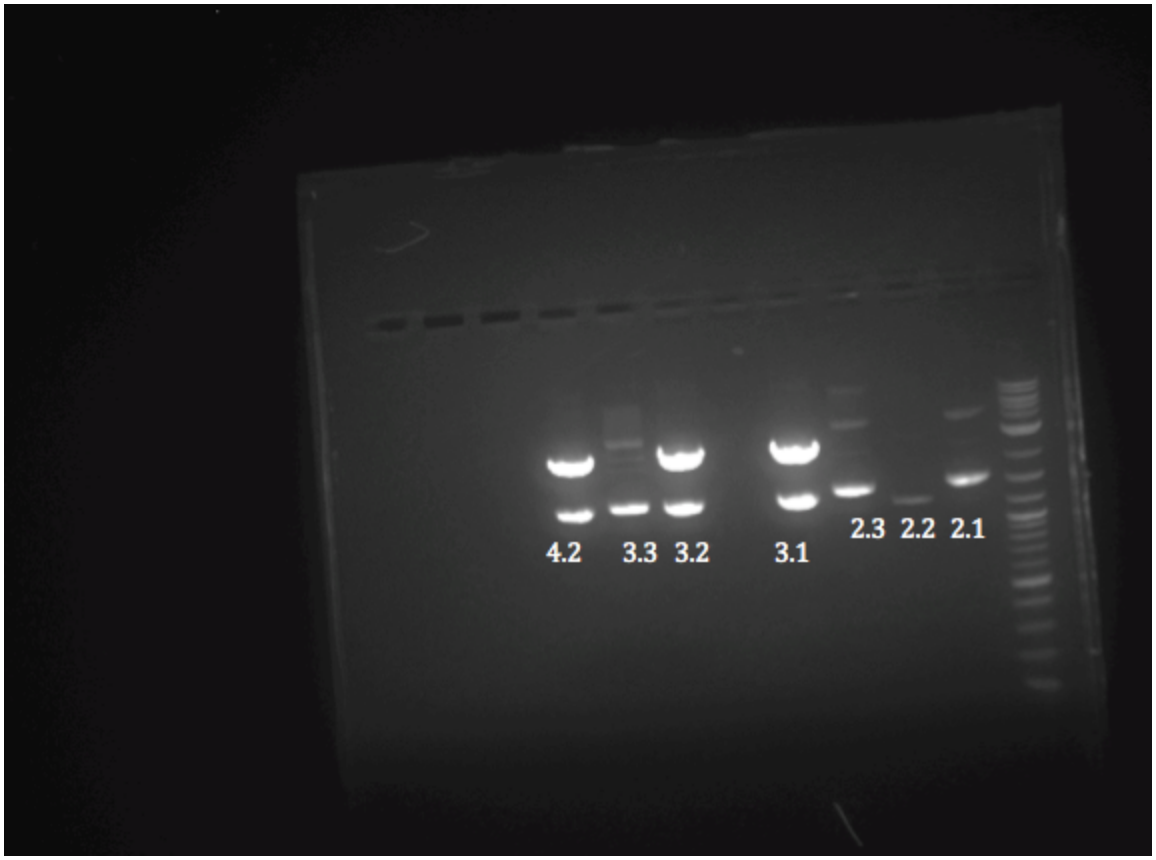
7/15/14
- Transformed the 8 ligations from 7/14/14. The 8 ligations are shown below:
T7+IK3+(4.1,4.2,4.3,4.4)
J23100+IK3+(4.1,4.2,4.3,4.4)
- Mini-prepped all twelve cultures started yesterday.

7/16/14
- Ran 8 PCRs, and then ran a gel. From left to right on the gel: 4.1 P1/P2, 4.2 P1/P2, 4.3 P1/P2, 4.4 P1/P2, 4.1 P1/P4, 4.2 P1/P4, 4.3 P1/P4, 4.4 P1/P4



- The 3 way ligations using linearized IK3 DNA, should be finished at 2200
- Digested the minipreps with X and P and ran a gel to see whether the samples were producing the correct band size. On the image, lanes from right to left read: 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, and 4.2

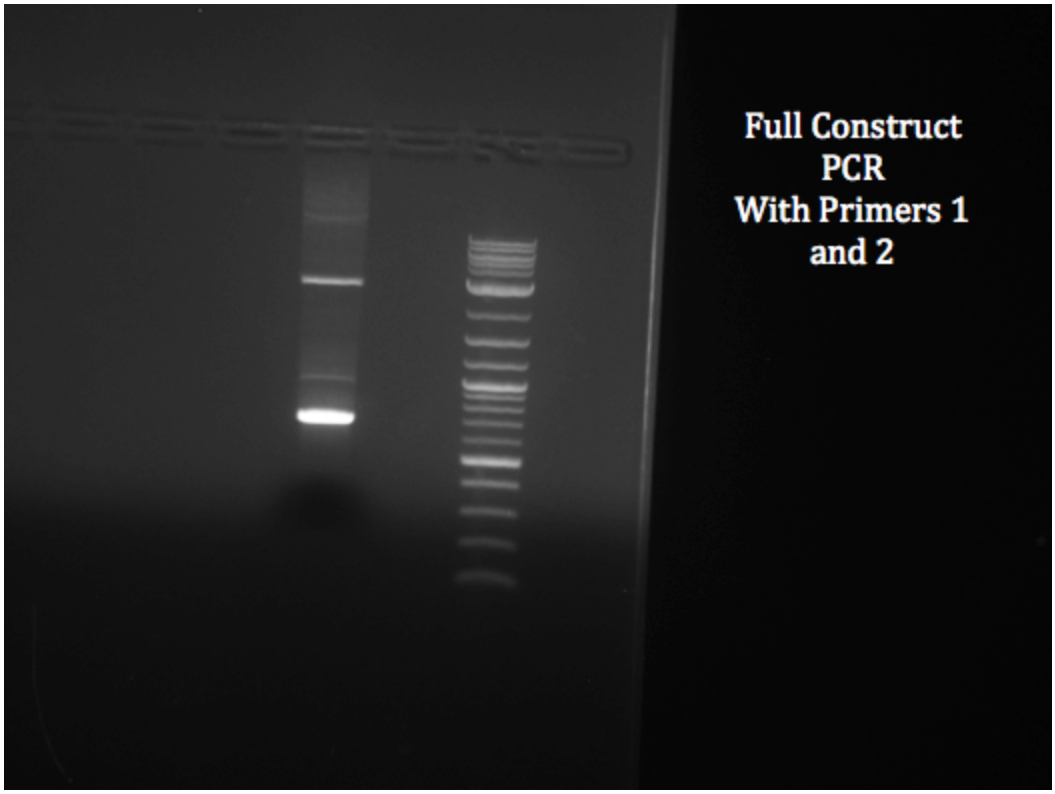
- We submitted the sequencing of 2.1, 3.1, 3.2, and 4.2 to confirm that the sequence is correct moving forward



7/17/14

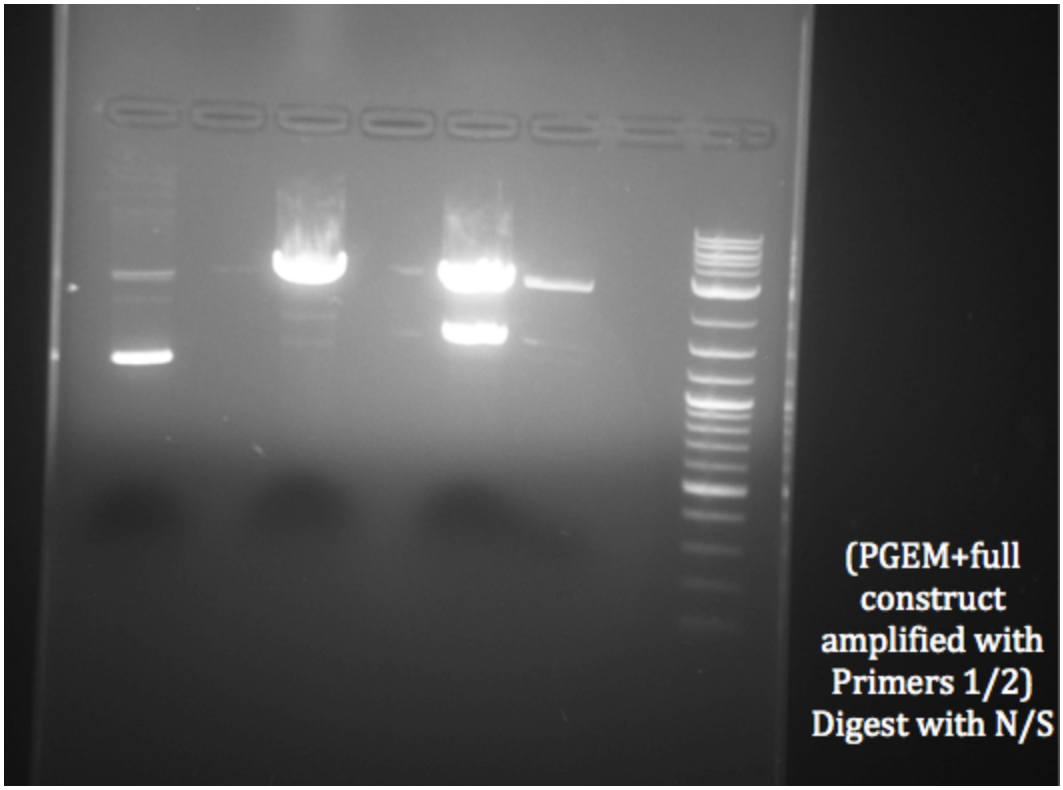
-Q5 PCR Amplification with primers 7&8 @ 62C

- Digested pGEM ligations(4.1,4.2,4.3,4.4) with N and S to see if the insert is in pGEM
- Ran the digest on a gel but there were no bands, so we started with the pGEM ligation again
- Did a Taq. PCR with P1/P2
- Ligated the gel extraction (from PCR P1/P2) with pGEM
- Conducted a 3A ligation of 1k3 (E/P), J23100 (S/P), and the digest products of 3.1, 3.2, and 4.2 (X/P)
- Started cultures of T7 and J06505 for Ocean and Ivor
- Ran out a gel to confirm Drew's digests from today (3.1,3.2,4.2 with 1k3) matched the rest of the teams'
- Ran a Q5 PCR amplification with primers 7 and 8 at 62C



7/18/14

- Made cultures from the replica plate of 4.1,4.2,4.3,4.4 pGEM ligations
- Ran digest products in a 1% agarose gel (loaded 50uL of samples)



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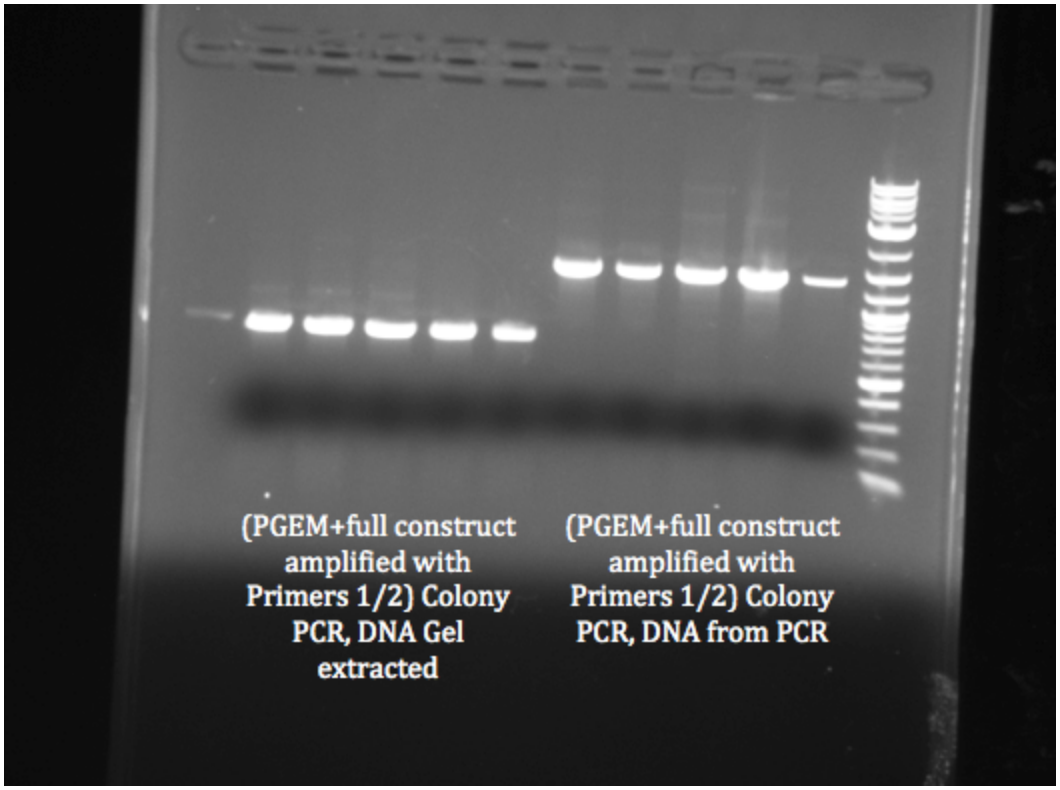
- Ligation did not work
- Transformed the 3A ligation (inserted 2uL of the ligation product) and allowed the ligation to sit overnight in case the ligation fails.

7/19/14
- The transformation of T7 + SYnth lin Ik3 ligation into DH5A was successful, but the transformation of J2300 failed
- Started an overnight culture and streaked a new plate of J2300 out
- pGEM ligations worked way better(more white colonies) using the DNA from the PCR compared to using the DNA from the gel extraction
-Made cultures, replica plates and colony PCR
-Run Colony PCR's in a gel(16 samples)



http://2014.igem.org/wiki/images/1/16/07-19-2014.png

7/20/14
- Repeated the ligation of J23100 with synt in 1k3
- Made BL21DE3 comp cells following lab protocol using CCMB80
-miniprepped all the pGEM ligations(Gel extracted)

7/21/14
-run gel of 7/19 PCR product (P7/P8)

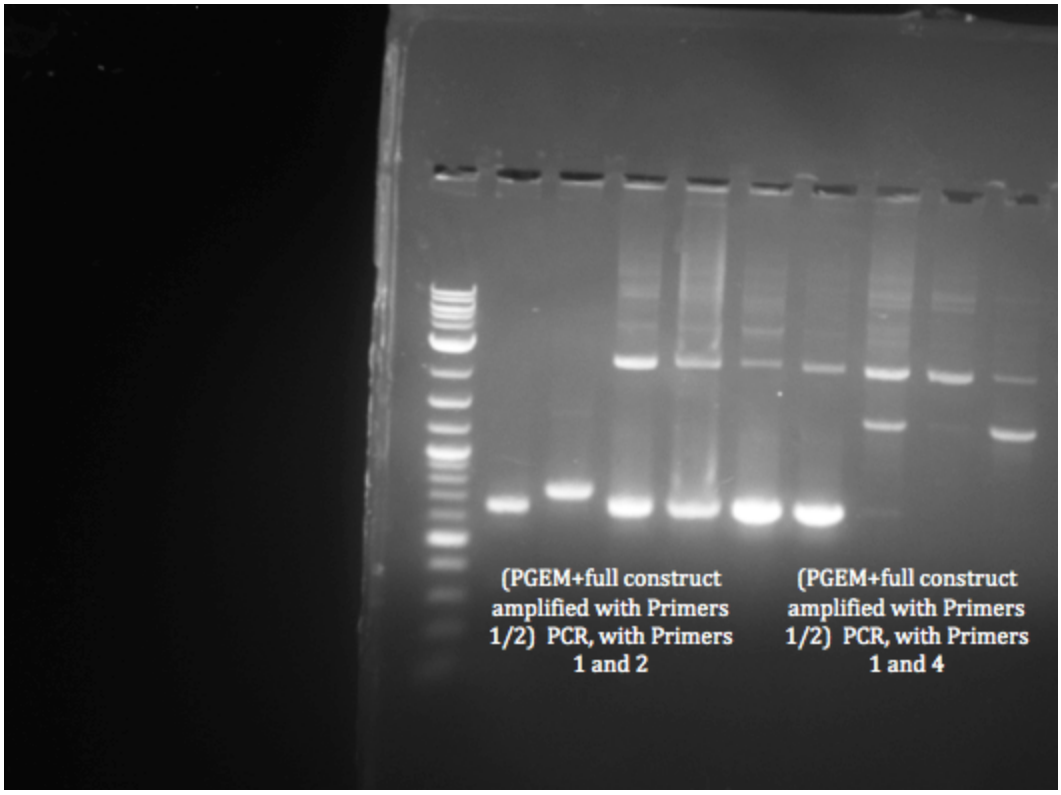
PCR product around 900 bp, positive confirmation

run 6 hr. digest of PCR product with N and S cut sites

transformed part K157008 into DH5alpha and BL21

- Transformed the T7 mini-prepped plasmid from the DH5A transformation on 7/18 into BL21DE3. Note that we heat-shocked for 10 seconds
- The transformation of J23100 failed again, so we digested new 4.2 with X and P to provide a new starting point for future ligations
- Ran gel of the 7/19 PCR product (P7/P8). The PCR product was around 900 bp, which is a positive confirmation.

- Ran a 6 hour digest of the PCR product (P7/P8) with N/S cut sites
- Transformed part K157008 into DH5alpha and BL21
-Run a a taq PCR with P1/P2 and P1/P4 in mini preps 3.4.5.6 from pgem ligation(these colonies were blue in the replica plate)



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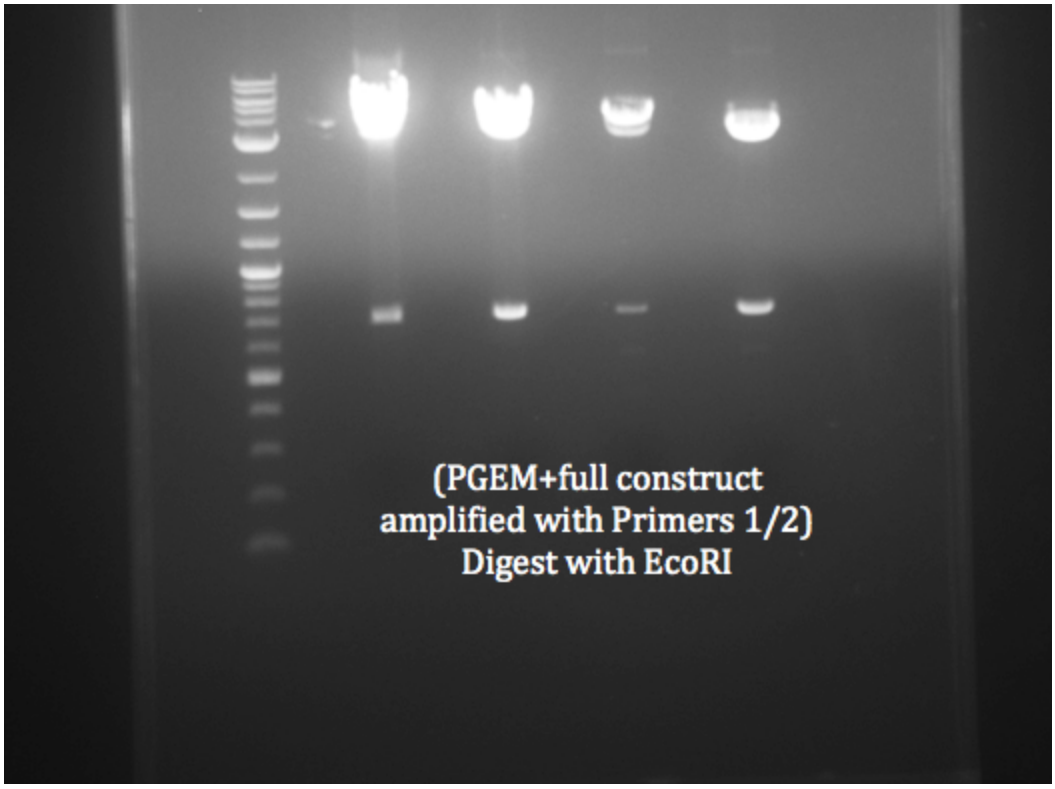
-Digested miniprep 3,4,5,6 from pGEM ligation(blue colonies) with EcoRI to see if the insert is present in pgem, left overnight

7/22/14
-Transformation of both comp cells worked
inoculate media DH5alpha with K157008, grow O/N
- The T7+ Full synthesis transformations worked with a 10 second heat shock time (both plates appeared to work). This means that the BL21 comp cells are competent!
- Started 3 cultures of T7+Synthesis in BL21 with IPTG induction at various times. Colony 1 was induced at time of inoculation, Colony 2 and 3 were induced 2 hours after inoculation.
- Ligated 4.2 (X/P), J23100 (E/S), and 1K3 (E/P) in four different concentrations (all ratios written as 1k3:j23100:synthesis):
1:3:3
2:2:2
1:4:2
1:2:4

- We will transform all of these ligations after 3 hours at RT and after overnight at 22.5C
- The transformation of K157008 into DH5alpha and BL21 worked, so we inoculated the media DH5alpha with K157008 and grew the cells overnight.
-Run gels with digest products pGEM+full construct(primers1/2) digest with EcoRI



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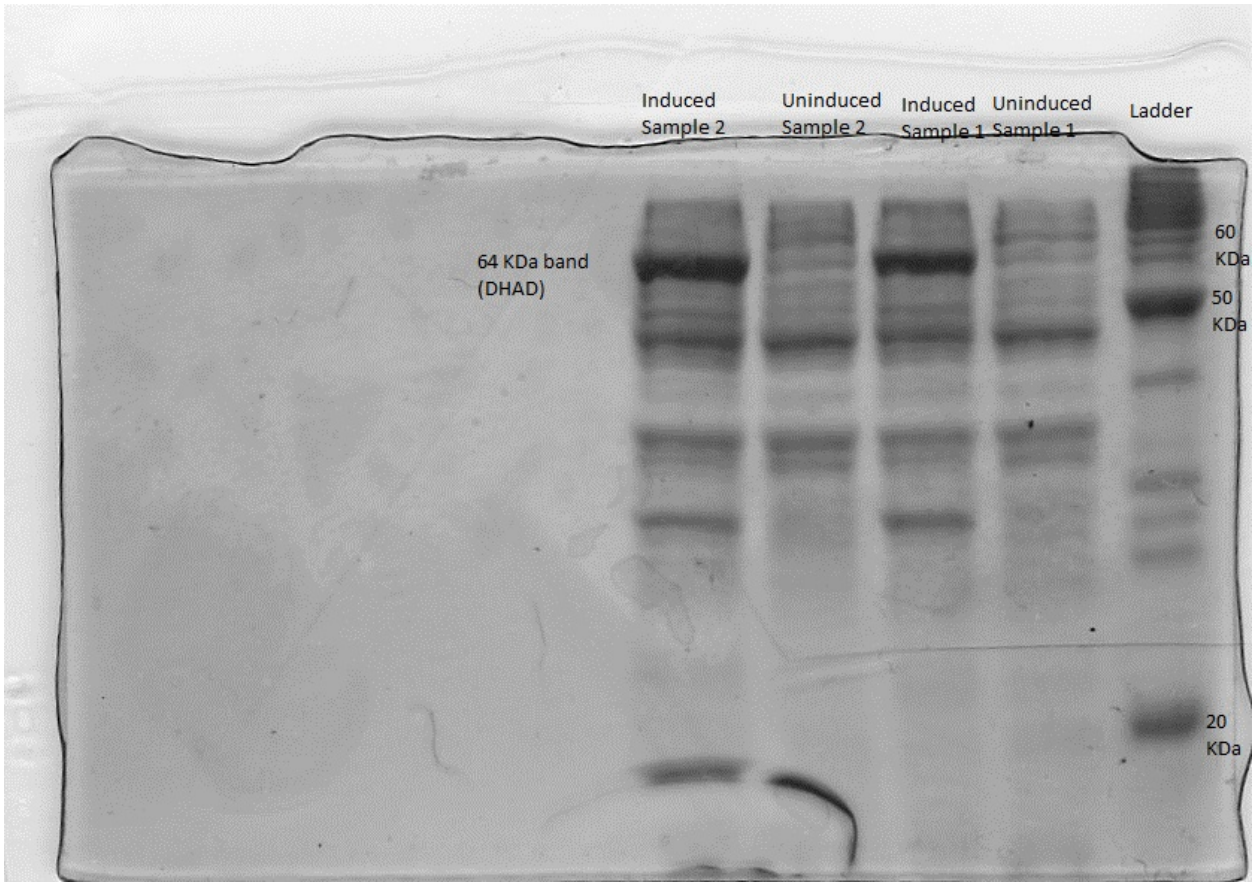
-Bands seems to be the right size ~660bp+~30(cut site)
-Digested mini preps from Gel Extraction with X/P (3,4,5,6)
-Ligated with promoters T7/J23100 and IK3 (8 ligations)

7/23/14
-mini-prep DH5alpha w/ K157008
digest mini-prepped K157008 with N and S
- The ligations were successful!
- 1:3:3 had no colonies but all other ratios appeared successful! 1:2:4 was the most successful, with the others being slightly less efficient (all less than 10 colonies - not bad for a 3A ligation regardless).
- At 8:30am the 3 colonies of T7 Full in the BL21 with IPTG did not appear red. The 3 colonies were placed back into the shaker.

- The J23100/4.2/1K3 ligations in four different concentrations were removed from the incubator and placed in the refrigerator.
- At 5:00pm the 3 colonies of T7 Full in the BL21 with IPTG did not appear red. The 3 colonies were placed back into the shaker.
- Mini-prepped DH5alpha with K157008 and digested with N/S
-Transformed 3 way ligation 9 8 ligation in total, 4 with 2 different promoters)

7/24/14
- Replica plated 8 colonies: 1 from 1:3:1, 2 from 2:2:2, and 5 from 1:2:4. We circled where colonies taken from on original plates and labeled where they were placed placed on the new replica plate. The replica plate was placed on the bottom shelf of the incubator and the original plates were put back into the fridge.
- Each replica plated colony was then placed in a tube of 3mL LB with 3uL of kanamycin. 8 tubes were labeled and placed into the shaker.
- Started 2 500 mL overnight cultures (1/20 innoculum) of Dr. Husts dihydroxyacid dehydratase strain to work on overexpressing and purifying the Dihydroxyacid dehydratase antigen used for later ELISAs. Cultures were induced (1mM IPTG) after 3 hours growth at OD600 .624 and .731 respectively for cultures 1 and 2. Uninduced samples were taken at this time point for comparison on a gel.
- Ran out digestion product from 7/23, phosphatased with Antarctic phosphatase for 1 hour, and ligated B7 with the K157008 digestion product
- Made cultures and Colony PCR from 3 way ligations

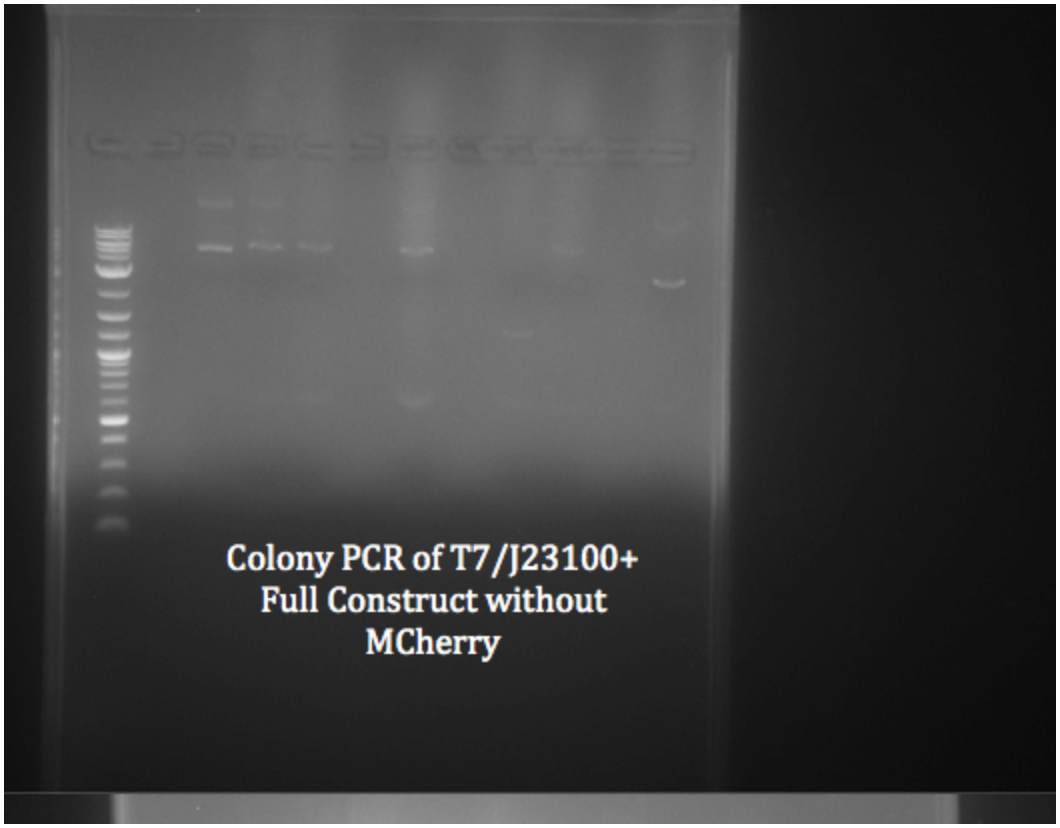
7/25/14
-transform ligation product into BL21
- Mini-prepped all potential J23100 + Synthesis parts, placed the minipreps in the freezer, and set up a Q5 PCR utilizing primers Vf2 and VR to screen potential ligation colonies from 7/24 to see if they were correct. We will run out a PCR next time in lab.
- Ran out 12.5% SDS-PAGE gel to visualize, utilizing the Benchmark protein ladder



http://2014.igem.org/wiki/images/a/a8/July_25.jpg

- Transformed the ligation product into BL21
-Minipreped plasmids from the successful transformations, stored in the freezer T7+5.1/5.2/6.1, J23100+3.1/3.2/6.1

7/26/14
-Run colony PCR of transformation plate from 7/25
-screen 10 colonies, Taq PCR, primers 7/8
-colonies 3, 5, 8, 10 were positive, grew up 3 mL cultures for mini-prep
- Ran a colony PCR of the transformation plate from 7/25
- Screened 10 colonies and did Taq PCR using primers 7/8. Colonies 3, 5, 8, and 10 were positive so we grew up 3mL cultures to be mini-prepped tomorrow.
-Did colony PCR again with Taq. from 3 way ligations (T7/J23100+Full construct without Mcherry)



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-There are ~700 faint bands that are the right size

7/27/14
- Mini-prepped colonies 3, 5, 8, and 10 from 7/26 and submitted sequencing data

7/28/14
- Gave Raoul and his team two samples of overexpressed pellets shown on the gel from 7/25. Raoul will carry out protein purification tonight with these samples.
- Ran out PCR from 7/25 of potential J23100 Synthesis ligations in 1k3.
- Repeated PCR on Kelly's minipreps from Friday using Taq instead of Q5 and an annealing temp of 55 and running out gel tonight (since we found out Q5 was contaminated)
-we got sequencing results back from the synthesis(p1/p2)+pGEM ligation, one of then 4.1(GE3) is right, the other three samples submitted have a non synonymous mutation that changes the Histidine for Arginine that may be beneficial later since the antibody we have already has a His tag
-transformed sequence verified plasmid(3GE)

7/29/2014
-Made cultures of the sequence verified plasmid(3GE) transformations
7/30/14

- Sequencing data showed that the construct was incorrect, will need to digest pgem with N/S and insert into RFC25, then ligate with the promoters t7/j23100
- Digested K157008 with N/S
-Miniprepped cultures from pgem sequence verified transformation
-Taq PCR from mini preps(sequence verified pgem ligations)
-Colony PCR from pgem ligations(with mutation)
-Started a culture from the original plate of the pgem ligation that was sequence verified(colony#3)

7/31/14
-Ran a gel of B7 pcr product digest and K157008 mini-prep digest
Gel extracted and gel purified both
Ligation of B7 (NS) and K157008 (NS)
- Ran a gel of B7 PCR product digest and K157008 miniprep digest
- Gel extracted and gel purified both the insert and vector
- Ligated B7 (N/S) and K157008 (N/S)